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is slightly higher in the yeast-fed animals than in the controls. In series 3, the agglutinin formation in both control and yeast-fed rabbits is the same, but in the controls the agglutinin remains in high concentration a longer time in the circulating blood of the control than in the yeast-fed animals. The lysin production is much higher in the control than in the yeast-fed animals.

From our work on the antibodies formed to sheep corpuscles we have been forced to conclude that yeast does not act by increasing antibody formation in the rabbit; in fact, it may even cause a reduction in the amount of antibodies formed as compared with those of the control animals.

Opsonins, the most active antibody infections, were not estimated, but the opsonin curve usually parallels the other antibody curves.

The laxative value of yeast was also found to be lacking, for there was no evidence of it in any of the yeast-fed rabbits of these three series. Their diet remained the same as that of the control animals in every respect save the one additional factor of yeast. We are unable for this reason to agree with Hawke's conclusions in regard to the rôle played by yeast in skin and other diseases; namely, a laxative value combined with a probable fixed effect on the intestinal tract.

The importance of any unknown substances we are not able to discuss. It may be that they are of great importance and play the determining rôle in the favorable effects obtained by yeast. As to alkaloids or other chemical substances, we have felt there is no need or evidence for their consideration.

CONCLUSIONS

The feeding of yeast has no stimulating effect on the production of antibodies to sheep blood in rabbits. The production of antibodies was even less than in controls.

There is no evidence of any effect of yeast on the gastro-intestinal tract of rabbits.

A BIOLOGIC CLASSIFICATION OF HEMOLYTIC STREPTOCOCCI

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INTRODUCTION — GENERAL CONSIDERATIONS

Streptococci as a group have long occupied a position of importance among pathogenic micro-organisms, and their importance has been enhanced in recent years. Their ubiquity and their pathogenicity place them in the front rank in the study of pathogenic bacteria. Yet surprisingly little is known of them. It is known that one large group of streptococci has the ability of hemolyzing blood, and that another large group has not this activity. Furthermore, it is known that their sugar-splitting activities are variable and that, by this criterion and by certain other cultural characteristics, subgroups exist. Aside from these facts very little is known.

A further interest in the streptococcus group, especially in the hemolytic members, was created by the high incidence of infection and healthy carriage of this organism at mobilization camps during the past two years. At some of the army camps and cantonments as high as 20-25% of healthy carriers of hemolytic streptococci were found.[1] These figures are much higher than those formerly obtained for civilian populations. Thus Smillie[2] found only a small percentage of carriers in a boys' school. A high carrier incidence has, however, been reported from many camps. Cumming, Spruit and Lynch[3] found 6% of healthy carriers at Fort Sam Houston; Fox and Hamburger[4] and Levy and Alexander[5] found 15% at Camp Zachary Taylor, and as high as 89% among regiments where bronchopneumonia was prevalent. Irons and Marine[6] report 70% of healthy carriers at Camp Custer.

That recently numerous individuals in civilian groups have harbored the hemolytic streptococcus is indicated by an examination of 76

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[1] Hamilton, C. D., and Havens, L. C.: Jour. Am. Med. Assn., 1919, 72, p. 272.

[2] Jour. Infect. Dis., 1917, 20, p. 45.

[3] Jour. Am. Med. Assn., 1918, 70, p. 1066.

[4] Jour. Am. Med. Assn., 1918, 70, p. 1827.

[5] Jour. Am. Med. Assn., 1918, 70, p. 1758.

[6] Jour. Am. Med. Assn., 70, p. 687.

students at a school in South Carolina whose throat cultures showed 22 to be carriers, or 29%.

This in turn raised a problem of epidemiologic importance. Were all these carriers to be considered a menace or were the majority harboring a relatively avirulent strain? A corollary of this was the determination of the biologic unity or multiplicity of hemolytic streptococci with a view to therapeutic and preventive measures.

Various attempts have been made in the past to differentiate the hemolytic streptococci. One of the best of these has been based on their carbohydrate reactions. Holman's[7] classification of 6 varieties according to their action on mannite and salicin has been widely followed, but, as will be shown later, only one or two of Holman's strains are commonly encountered.

Another basis of differentiation has been their hemolytic activity. Gay[8] associates increased virulence with increased hemolytic activity and suggests hemolytic tests, using standard amounts of red cell suspensions and determining the amount of a given culture which produces complete hemolysis. The hemolytic activity of streptococci will be discussed later in this report. At this point it can be said that it is a recognized fact that streptococci vary greatly in virulence, not only among different strains, but also with the same strain, depending on the environment.

A third method of classification, and one which has given rise to the most progress in the study of other micro-organisms, is based on biologic reactions. Kinsella and Swift,[9] from a study of a limited number of strains, by means of the complement fixation test, came to the conclusion that they were identical. Similarly, Howell[10] finds a low specificity by complement fixation for this group. Davis,[11] studying the anaphylactic reactions of hemolytic streptococci, found that a number of different strains interacted. It should be pointed out, however, that in these experiments only a limited number of different cultures were used. Furthermore, the delicacy of the complement fixation reaction and of anaphylactic tests is admittedly not as great nor their specificity as reliable as in agglutination tests, for example, where high dilutions of serum can readily be used.

[7] Jour. Med. Research, 1916, 34, p. 377.

[8] Jour. Lab. and Clin. Med., 1918, 3.

[9] Jour. Exper. Med., 1918, 28, p. 169.

[10] Jour. Infect. Dis., 1918, 22, p. 230.

[11] Jour. Infect. Dis., 1917, 21, p. 308.

A further step in the differentiation of hemolytic streptococci has been made by Avery and Cullen.[12] They have studied the final H-ion concentration of cultures of strains of bovine and human origin, and have found a constant and remarkable difference between strains from these two sources. Human strains produced constantly less acid than the bovine strains, i. e., the final H-ion concentration was consistently higher with the latter than with the former group.

Recently it has been shown in a preliminary report that hemolytic streptococci apparently differ among themselves in respect to their serologic characteristics as judged by agglutination tests, bactericidal experiments and protective properties for animals, especially mice. It was shown that a serum high in agglutinins could be produced in rabbits and that about 55% of a series of 110 strains fell in one group whereas the rest showed serologic differences. The purpose of this paper is to test the work presented in the preliminary report, and to describe further experiments in connection with the serologic grouping of the hemolytic streptococci.

Source of Strains.—The basis of these experiments is furnished by a series of strains from different sources which include throat cultures from healthy carriers, from cases of acute bronchitis, from measles patients, and from cases of tonsillitis and sore throat; sputum from pneumonia and lung cultures at autopsy; empyema; gunshot wounds, and one case of renal infection. The number of cultures from each source is presented in the following table:

TABLE 1

SHOWING NUMBER OF CULTURES FROM EACH SOURCE

Throat cultures—all sources		179
Healthy carriers	80	
Acute bronchitis	60	
Measles	9	
Tonsillitis and sore throat	30	
Pneumonia—sputum		20
necropsy		21
Empyema		67
Gunshot wounds		4
Renal infection		1
Total		292

In addition, thanks are due to Dr. O. T. Avery of the Rockefeller Institute, who kindly furnished a few representative strains.

Cultures were in all cases first streaked on a blood-agar plate and characteristic colonies showing a definite, clear hemolytic zone at the end of 18-24 hours' incubation were then fished and grown on blood-agar slants. A smear was examined microscopically for morphology, and the slant was kept in the icebox for a stock culture.

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[12] Jour. Exper. Med., 1919, 29, p. 215.

These strains may be considered quite representative, since they were obtained not from the population of a single community, but from arrivals in the camp (Wadsworth) from widely scattered parts of the country, and from patients from many camps. Furthermore, the pathologic conditions represented by the series is quite inclusive.

Cultural Characteristics.—The cultural characteristics of hemolytic streptococci have previously been described in detail, and it is necessary only to mention certain of their growth activities which bear on their differentiation. The criterion of any strain of hemolytic streptococcus is, of course, its ability to hemolyze red blood corpuscles. Furthermore, the blood is not only laked, but is destroyed still further. The hemoglobin is broken down so that on a blood-agar plate there is a clear zone containing no pigment. Another point in the differentiation of these organisms by means of their hemolytic action is that the zone is clear-cut—the hemolysin acts completely, if it acts at all.

Among different strains, the hemolytic action varies markedly. In the first place, some colonies produce a wide zone, others a narrow zone, merely a rim around the colony. Again the amount of a 24-hour broth culture which is required to completely hemolyze a given amount of blood (0.5 c c of a 5% suspension of human cells) varies considerably, not only between different strains, but also from time to time in the same strain. Some strains completely hemolyze this amount of blood in 0.05 c c amounts, others do not produce complete hemolysis in 0.5 c c amounts.

As remarked, the hemolytic action of any particular strain seems to vary with its virulence. Streptococci rapidly decrease in virulence with growth on artificial mediums. At the same time its hemolytic action diminishes. A strain of hemolytic streptococcus grown for 2-3 weeks on blood agar possesses only feeble hemolytic power and such a culture, injected into mice, may fail to kill in 2 c c amounts. Its virulence, however, can be quickly raised so that after 6 passages, in the case of 3 strains studied, 0.1 c c of a 24-hour culture killed invariably in less than 24 hours. Along with increased virulence its hemolytic power rises. Its virulence is lost with equal rapidity, cultivation for from 7-10 days on artificial mediums again reducing it to its former avirulence and its feeble hemolytic activity.

Carbohydrate tests with different strains of hemolytic streptococci, as mentioned, have been used for their differentiation. However, of the 6 groups which have been determined by this method only two are commonly met with. These are S. anginosus and S. pyogenes, the former splitting only lactose, the latter acting on salicin as well, neither affecting mannite. Of 120 strains of this series studied in this way, 80% split only lactose, the other 20% acted on both lactose and salicin. In this series, no evidence of any relation of the action on sugars to the hemolytic action, to the serum reactions, or to the virulence of the organisms could be found.

It can be seen from the preceding observations that no method was at hand for satisfactorily differentiating hemolytic streptococci. Its lack has been felt. It is known that animals can be immunized against individual strains of streptococci, but this has acquired no practical importance in spite of repeated attempts. No adequate basis for determining different strains has been available. The method in practice for producing antistreptococcus serum has been to collect as large a number of different cultures as possible and to trust that they constituted a fairly complete representation of the streptococci. Consequently, the serum was either of low titer due to its great polyvalency or was useless because it did not contain antibodies against the particular strain causing the infection. The same criticism applies equally to the vaccines made in this way. Hence, a method for "typing" hemolytic streptococci is for this reason alone of practical importance.

Serologic Experiments.—Three cultures were chosen at random from the series and were used for immunizing rabbits. The cultures were grown for from 3-4 weeks on blood agar and were avirulent for rabbits. The injections were made with the unheated centrifuged sediment of serum broth cultures resuspended in normal salt solution. The avirulent living organisms were injected intravenously in increasing amounts, on 3 successive days, followed by a 3-day interval. Nine such injections were, in most cases, sufficient to obtain an agglutinin titer of 5,000-10,000.

A plain or glucose broth culture of hemolytic streptococcus has a tendency to clump spontaneously. It was found, however, that plain broth to which 10% defibrinated blood has been added gives a medium in which the organisms grow rapidly and which in 12-16 hours shows a uniform, even turbidity. The blood is only very slightly hemolyzed by old stock cultures in this length of time, the hemolysis, such as there is, being confined to the butt of the tube, leaving the upper portion free from hemoglobin. Tubes were set up containing dilutions of serum from 1:10-1:4,860 in 0.5 c c amounts and 0.5 c c of culture making 1 c c, the total amount. A control tube was used with each culture containing 0.5 c c salt solution and 0.5 c c culture. The tubes were incubated at 37 C. for 4 hours and then placed in the icebox over night and the final reading made the next morning, the titer given in each case being the highest dilution of serum in which complete agglutination took place.

The serum obtained from rabbits injected, as outlined, with these 3 strains was used in agglutination tests with all the cultures of the series. It was soon found that these 3 serums were identical, that is, strains which agglutinated with one, agglutinated with the other two, and strains which failed to agglutinate with one were not agglutinated by the others. Consequently the serums were pooled. With this serum 139 strains, or 47%, of the 292 members of the series were agglutinated in dilutions of 1:1,000 or higher. The other 153 strains failed to agglutinate, or did so only in low dilution—never higher than 1:100, with the exception of a small series which will be discussed in detail later.

One of these strains which failed to be agglutinated was then used for the production of an immune rabbit serum, using the technic employed for the first serum. With this serum 54, or 19%, were found to agglutinate. With the exception of the series mentioned above, no instances of cross-agglutination were found. Thus, according to their agglutination reactions, we now have two groups which are distinct and well defined. They include together 191, or 66%, of the 292 members of the series.

A third group comprising 79 members of the series, or 27%, was obtained by similar methods. This group, like the others, contains a few strains which show evidence of a certain interrelation as judged by cross-agglutination, but the majority are distinct.

It appears, then, that by means of agglutination tests three distinct groups of the hemolytic streptococci are established, constituting 93%

of this series of 292 strains. The first group is much the largest (47%), the second group contains 19%, and the third, 27%. The remaining 22 strains, or 7% of the series, evidently is a heterogeneous group, possessing different biologic characteristics. At this time no evidence can be presented to show that this group can be satisfactorily subdivided.

In order to determine whether other immune bodies, aside from agglutinins, corroborated this grouping, bactericidal tests were made on a considerable number of strains. Each test was made with 4 tubes: To each tube was added 0.5 c c of a blood broth culture of the particular strain to be tested and 0.1 c c of fresh guinea-pig serum as complement. To the first tube was added 0.5 c c group 1 serum, to the second tube 0.5 c c group 2 serum and to the third tube 0.5 c c serum 3. The fourth tube received 0.5 c c salt solution as a control. The tubes were then incubated for 4 hours at 37 C. and 0.1 c c of the mixture in each tube was plated in blood agar. After 24 hours' incubation, the number of colonies were counted. The following table shows a comparison of bactericidal tests with agglutinations.

TABLE 2

COMPARISON OF AGGLUTINATION TESTS AND BACTERICIDAL EXPERIMENTS

Number of Culture	Number of Colonies*				Agglutination Tests		
	Serum 1	Serum 2	Serum 3	Salt Solution	Serum 1	Serum 2	Serum 3
159	0	1,000	1,000	1,000	4,860	180	10
161	0	200	100	200	4,860	20	40
170	0	100	100	200	4,860	180	60
164	0	200	200	200	4,860	20	10
163	50	1,000	1,000	1,000	4,860	20	60
169	25	1,500	2,000	2,000	4,860	180	60
172	100	1,000	1,000	2,000	4,860	10	60
186	0	100	200	200	4,860	60	20
79	10	200	400	500	4,860	180	10
3	0	500	500	500	4,860	10	10
33	10	500	1,000	1,000	10,000	180	10
50	100	2,000	2,000	2,000	4,860	10	10
124	0	500	400	400	5,000	60	180
117	500	10	500	500	10	4,860	20
206	200	0	200	200	60	4,860	60
51	1,000	10	1,000	1,000	10	4,860	10
67	200	20	300	500	180	4,860	10
129	1,000	100	2,000	2,000	1,620	4,860	10
152	200	0	200	200	60	4,860	20
180	200	25	200	250	180	4,860	10
245	100	0	100	200	10	4,860	20
121	100	20	200	200	180	1,620	540
118	200	0	500	500	10	4,860	60
196	1,000	0	500	1,000	10	4,860	10
135	1,000	1,000	10	1,000	10	10	4,860
183	1,000	2,000	50	2,000	540	60	4,860
178	2,000	2,000	100	2,000	10	60	4,860
151	1,000	500	10	2,000	180	10	4,860
211	200	200	0	200	20	10	4,860
209	100	100	10	200	10	10	4,860
218	200	100	0	200	60	180	4,860
248	1,000	2,000	100	2,000	10	10	4,860
34	1,000	1,000	10	2,000	20	60	4,860
16	500	500	50	500	50	10	4,860
199	200	200	0	500	1,620	1,620	4,860

* After 24 hours incubation on a blood-agar plate inoculated with 0.1 c c of serum culture incubated for 4 hours.

It can be seen from the table that the bactericidal experiments agree with the agglutination tests; that is, strains were killed by the same serum with which they agglutinated, but not by serum of another group.

It was mentioned that a number of strains seemed to be interrelated in that, while with one serum they agglutinated in dilutions of 1: 5,000, they also agglutinated with serum of another group in quite high dilution, as high as 1: 1,500 in a few instances. These strains are interestng in that they furnish evidence of a lack of complete and clear cut differentiation between the groups, such as exists between the pneumococcus groups. One group appears to shade into another somewhat as the different strains of meningococcus are interrelated. The strains which agglutinated higher than 1: 100 with more than one serum are summarized in table 3, together with their agglutination titer with each serum. With serum of the titer used in these tests (1: 5,000 or 10,000) a positive agglutination in dilutions of less than 1: 100 was regarded as nonspecific.

TABLE 3

INTERRELATED STRAINS SHOWING AGGLUTINATION TITER WITH EACH GROUP SERUM

Number of Culture	Serum 1	Serum 2	Serum 3
3	10,000	10	180
10	4,860	10	180
33	10,000	180	10
35	4,860	10	180
60	3,240	540	180
79	4,860	180	10
107	3,240	10	180
124	5,000	60	180
146	4,860	180	180
159	4,860	180	10
154	3,240	180	60
162	4,860	10	540
179	4,860	60	180
191	4,860	1,620	10
170	4,860	180	60
209	4,860	540	10
58	180	4,860	60
129	1,620	4,860	10
121	180	1,620	540
170	1,620	4,860	20
180	180	4,860	10
235	540	4,860	10
96	180	4,860	10
207	20	4,860	180
199	1,620	1,620	4,860
95	540	540	4,860
125	180	180	4,860
151	180	10	4,860
183	540	60	4,860

An analysis of table 3 shows that 29 strains indicate by their agglutination an interrelation between the groups. Sixteen members of group 1 which are agglutinated in high dilution by their homologous serum were agglutinated in dilutions of 1 : 100 or higher by one or both of the other two serums. Seven were agglutinated only by serum 2 in addition to the serum of their own group in dilutions varying from 180-1,620 and 7 only by serum 3 in dilutions of 180-540. Two strains, 60 and 146, were agglutinated by both serums 2 and 3 in dilutions above 1 : 100. Eight strains of group 2 showed some relation to the other 2 groups; 6 only to group 1, and 1 only to group 3. Strain 121, in addition to agglutinating in its homologous serum up to 1 : 1,620, agglutinated in serum 1, 1 : 180, and in serum 3, 1 : 540. Five members of group 3 were agglutinated by serums 1 and 2 in dilutions above .100. Two were agglutinated by serum 1, 1 : 180 and 1 : 540, respectively, and 3 equally by serums 1 and 2, 1 : 1,620, 1 : 540 and 1 : 180.

Thus 29, or about 10%, of the 292 strains gave evidence from agglutination tests of some relation to other groups besides the one with the serum of which they were agglutinated in highest dilution. This relationship is borne out to some extent by bactericidal experiments; as, for example, in table 2, culture 79, which is agglutinated by serum 1, 1 : 4,860, by serum 2, 1 : 180 and serum 2, 1 : 10, shows by bactericidal tests, 10 colonies with serum 1, 200 with serum 2, while serum 3 showed scarcely more effect than normal salt solution, namely, 400 colonies. Cultures 33, 124, 129, 183 and 199 are further examples. This relationship is not, however, borne out in all cases by bactericidal tests, as for example, strain 121, which was agglutinated by serum 3, 1 : 540, gave as many colonies in the bactericidal test with this serum as the control. Cultures 159, 170, 180, and 151 are further examples.

While agglutination tests and bactericidal experiments give this evidence of interrelationship among groups, protective experiments with mice fail to show it. Only the serum which possesses a titer above 1,000 for the strain used for injection seems to possess sufficient protective properties to prevent death with a lethal dose. These experiments will be described in detail.

From several points of view a study of this series of hemolytic streptococci grouped according to the character of the infection or the pathologic condition from which the strains were isolated is interesting and instructive. The following tables show the groupings of these strains according to the character of the infection. Table 4 shows the groupings of the whole series for comparison with the following tables.

TABLE 4

SOURCES

Total Strains from all Sources:			5. Pneumonia—Sputum:		
Group 1	137	47%	Group 1	8	40%
Group 2	54	19%	Group 2	4	20%
Group 3	79	27%	Group 3	6	30%
Group 4	22	7%	Group 4	2	10%
Total	—292		Total	—20	
1. Throat Cultures—Healthy Carriers:			6. Pneumonia—Necropsy:		
Group 1	37	46%	Group 1	20	95%
Group 2	16	20%	Group 2	0	0%
Group 3	18	22%	Group 3	1	5%
Group 4	9	12%	Group 4	0	0%
Total	—80		Total	—21	
2. Throat Cultures—Acute Bronchitis:			7. Empyema:		
Group 1	29	48%	Group 1	28	42%
Group 2	12	20%	Group 2	15	22%
Group 3	14	23%	Group 3	20	30%
Group 4	5	9%	Group 4	4	6%
Total	—60		Total	—67	
3. Throat Cultures—Measles:			8. Gunshot Wounds:		
Group 1	5	55%	Group 1	0	0%
Group 2	4	45%	Group 2	0	0%
Group 3	0		Group 3	3	75%
Group 4	0		Group 4	1	25%
Total	—9		Total	—4	
4. Throat Cultures—Tonsillitis and Sore Throat:			9. Renal Infection:		
Group 1	9	30%	Group 1	1	
Group 2	3	10%			
Group 3	17	57%			
Group 4	1	3%			
Total	—30				

It will be seen, in the first place, that the group of 80 strains from carriers compares very closely with the percentages of the entire series. This would appear to be of epidemiologic importance in that healthy carriers are a source for any and all of the pathologic conditions that are caused by hemolytic streptococci. From the small evidence at hand the impression has been gained that carriers of these organisms rarely develop an infection, at least with the particular strain which they harbor.

The strains obtained from cases of bronchopneumonia, while small, are exceptionally interesting. From the table it is evident that the percentages of each group follow approximately the percentages of the whole series. No one group seems to be associated predominantly with this condition. This is the case with antemortem specimens. Postmortem cultures, however, show a great contrast to the cultures from sputum. Of 21 strains obtained from necropsies, all but one were found to belong to the first group, the other one falling in group 3. Although it is hazardous to draw conclusions from a small series such as this, yet the contrast between the antemortem and the postmortem cultures is so marked that it would appear to indicate a special virulence for group 1 organisms. While all the groups may cause pneumonia in a proportion relative to their frequency, it would seem that the prognosis is much more favorable in group 2 and 3 infections than in the case of group 1. A further point in connection with streptococcus pneumonia is of interest. It appears that no one group predominates in the causation of empyema. This condition is as likely to develop following infection with one group as with another. The proportions of the groups in the strains obtained from empyema correspond closely with the groups in pneumonic sputum.

The series of cultures from cases of tonsillitis and sore throat is also of interest. It would seem that group 3 is more commonly found in this condition than any other group. But most of these cases occurred in one ward, in fact, all the group 3 cases developed in this one ward, and very probably spread from one source. However this may be, it is interesting from an epidemiologic viewpoint. As a matter of fact, in most epidemics in the past, a common source of the infection has been suspected, but it has not been susceptible of proof. In this small epidemic which occurred in a hospital ward, all but three of the cases were due to a group 3 organism. However, the fact that there were three cases due to other groups — two to group 1 and one to group 4 — shows that while most of the cases were due to one source, in all probability not all were developed from the same strain. The mere finding of hemolytic streptococci does not implicate them in the causation of an epidemic, for they may belong to a different group from the one causing the epidemic.

Another point in connection with the epidemiology of hemolytic streptococcus infections is the series of strains isolated from wounds which, while only 4 in number, show only one case which falls into group 4 — the heterogeneous group of nonagglutinating strains. A priori, it might seem that most of these strains which infect wounds would fall in a different category from those which are more obviously from a human source, but evidently these organisms, carried into the wound, usually on bits of clothing, belong to the same groups as the strains found in other infections.

A summary of the entire series giving the number of strains according to their source and the number and percentage of each group is collected in table 5.

TABLE 5

SUMMARY OF 292 STRAINS OF HEMOLYTIC STREPTOCOCCI GROUPED BY AGGLUTINATION AND PATHOLOGIC SOURCE

Pathologic Condition from which Strains Were Isolated	Group 1		Group 2		Group 3		Group 4	
	No. Strains	Per Cent.	No. Strains	Per Cent.	No. Strains	Per Cent.	No. Strains	Per Cent.
Throat Culture:								
Healthy carriers........	37	46	16	20	18	22	9	12
Acute bronchitis........	29	48	12	20	14	23	5	9
Measles.................	5	55	4	45	0	0	0	0
Tonsillitis and sore throat................	9	30	3	10	17	57	1	3
Pneumonia:								
Sputum.................	8	40	4	20	6	30	2	10
Necropsy................	20	95	0	0	1	5	0	0
Empyema.................	28	42	15	22	20	30	4	6
Gunshot wound...........	0	0	0	0	3	75	1	25
Renal infection..........	1	100	0	0	0	0	0	0
Total.................	137	47	54	19	79	27	22	7

PROTECTIVE EXPERIMENTS

It can be assumed from the experiments previously described that hemolytic streptococci fall into certain quite specific groups and that, in vitro, they are killed by their homologous serum, but not by the serum of other groups. It remains to determine whether they react in the same manner in the animal body and whether increased virulence is accompanied by any change in the serologic reactions of the organism.

In the experiments about to be described the serum used was prepared in the same way as for the agglutination and bactericidal tests, namely, by injecting rabbits with live avirulent organisms, bleeding them when the agglutinin titer for the homologous strain reached 5,000-10,000. Mice were used for the tests and the strains were increased in virulence by passage through mice until 0.1-0.25 c c of a 24-hour broth culture killed in 24 hours. Twelve mice were injected intraperitoneally with the serum prepared in this way, four with the serum of each group, in varying amounts, and at the same time with twice the minimum lethal dose of the corresponding organism. No evidence of any protection by the serum was obtained, whether injected some time before the streptococci or at the same time. Two possibilities were to be considered in consequence. Either the agglutinin titer gave a wrong impression of the protective strength of the serum or the individual strains became altered in their biologic properties as their virulence increased. Both possibilities might be the case. It seemed more probable, however, that the agglutinin content of the serum was not a criterion of the amount of protective action which it possessed. Evidence that this was the case was furnished by the fact that a given strain showed no difference in its agglutinating properties after passage through mice. It gave the same titer with each group serum after several passages through mice as it did when it had been grown for a long period on artificial medium.

The method of producing the serum was consequently modified. Rabbits were given 3 series of injections with avirulent organisms as before, on 3 successive days, with 3-day intervals, making 9 injections in all. Instead of now testing and bleeding as in the case with the serum used for agglutinations, the rabbits received 2 more series of injections—6 on successive days divided by a 3-day interval—of strains which had recently been passed through mice and which were highly virulent for this animal. Using serum prepared in this way the following experiments were carried out:

Mouse 1: (Control.) 0.1 c c 24-hour blood-broth culture 197 (group 1). Dead in 24 hours.

Mouse 2: 0.2 c c 24-hour blood-broth culture of strain 197 + 0.5 c c group 1 serum. Normal 24 hours later.

Mouse 3: 1 c c group 1 serum followed 1 hour later by 0.2 c c 197. Inactive and sick 18 hours later. Peritoneal puncture shows many polymorphonuclear leukocytes loaded with streptococci. Given 0.5 group 1 serum. Normal 24 hours later.

Mouse 4: (Control.) 0.1 c c blood-broth culture of strain 209 (group 1). Dead 18 hours later.

Mouse 5: 0.2 c c strain 209 + 0.5 c c serum 1. Normal 24 hours later.

Mouse 6: (Control.) 0.1 c c 24-hour blood broth culture of strain 170 (group 2). Dead in 24 hours.

Mouse 7: 0.2 c c strain 170 + 0.5 c c serum 2. Normal 24 hours later.

Mouse 8: (Control.) 0.1 c c 24-hour blood-broth culture of strain 135 (group 3). Dead 24 hours later.

Mouse 9: 0.2 c c strain 136 + 0.5 c c serum 3. Normal 24 hours later.

The protocols show that it is possible to raise the protective properties of antistreptococcus serum to a point where it has some value for mice, at least. In the case of mouse 3 the serum apparently had a distinct therapeutic value even after the infection had gained headway and had become systemic.

The following protocols further corroborate the specificity of these 3 groups of hemolytic streptococci. Mice were inoculated intraperitoneally with 2 lethal doses of each strain together with 0.5 c c of each group serum.

Mouse 1: (Control.) 0.1 c c 24-hour blood-broth culture of strain 209 (group 1). Dead in 20 hours.

Mouse 2: 0.2 c c 24-hour blood-broth culture 209 (group 1) + 0.5 c c serum 1. Normal 24 hours later.

Mouse 3: 0.2 c c culture 209 (group 1) + 0.5 c c group 2 serum. Dead in 18 hours.

Mouse 4: 0.2 c c culture 209 (group 1) + 0.5 c c group 3 serum. Dead in 24 hours.

Mouse 5: (Control.) 0.1 c c 24-hour blood-broth culture of strain 170 (group 2). Dead in 18 8hours.

Mouse 6: 0.2 c c strain 170 (group 2) + 0.5 c c group 1 serum. Dead in 24 hours.

Mouse 7: 0.2 c c culture 170 + 0.5 c c group 2 serum. Normal 24 hours later.

Mouse 8: 0.2 c c culture 170 + 0.5 c c group 3 serum. Dead in 18 hours.

Mouse 9: (Control.) 0.1 c c 24-hour blood-broth culture of strain 135 (group 3). Dead in 24 hours.

Mouse 10: 0.2 c c culture 135 (group 3) + 0.5 c c group 1 serum. Dead in 24 hours.

Mouse 11: 0.2 c c culture 135 (group 3) + 0.5 c c group 2 serum. Sick in 24 hours. Dead in 28 hours.

Mouse 12: 0.2 c c culture 135 (group 3) + 0.5 c c group 3 serum. Normal 24 hours later.

In these experiments it is seen that only the homologous serum protected against infection. In each case the mouse injected with the serum corresponding to the group to which the strain inoculated belongs, lived. All the other mice died. However, each mouse received two fatal doses of the culture. In order to determine if the serum of other groups possessed protective properties in any significant amount whatever, the following series of mice were injected with serum and culture mixtures, but the mice receiving heterologous serum were given only one lethal dose instead of two, which the mice injected with homologous serum received.

Mouse 1: (Control.) 0.1 c c strain 209 (group 1) intraperitoneally. Dead in 24 hours.

Mouse 2: 0.2 c c strain 209 (group 1) + 0.5 c c group 1 serum. Normal 24 hours later.

Mouse 3: 0.1 c c strain 209 (group 1) + 0.5 c c group 2 serum. Dead in 24 hours.

Mouse 4: 0.1 c c strain 209 (group 1) + 0.5 c c group 3 serum. Dead in 20 hours.

Mouse 5: (Control.) 0.1 c c strain 170 intraperitoneally. Dead in 18 hours.

Mouse 6: 0.1 c c strain 170 (group 2) + 0.5 c c group 1 serum. Dead in 18 hours.

Mouse 7: 0.2 c c strain 170 (group 2) + 0.5 c c group 2 serum. Normal 24 hours later.

Mouse 8: 0.1 c c strain 170 (group 2) + 0.5 c c group 3 serum. Dead in 30 hours.

Mouse 9: (Control.) 0.1 c c strain 135 (group 3) intraperitoneally. Dead in 26 hours.

Mouse 10: 0.1 c c strain 135 + 0.5 c c group 1 serum. Dead in 24 hours.

Mouse 11: 0.1 c c strain 135 (group 3) + 0.5 c c group 2 serum. Dead in 24 hours.

Mouse 12: 0.2 c c strain 135 (group 3) + 0.5 c c group 3 serum. Normal 24 hours later.

As in the preceding experiments, the culture injected with heterologous serum killed the mice as rapidly as in the case of the control mice which received no serum. No evidence of any protection by the heterologous serum was obtained. Thus, in view of these experiments, the specificity of the 3 groups of hemolytic streptococci which have been described is borne out by protection experiments in mice as well as, in vitro, by agglutination and bactericidal tests.

DISCUSSION

Many species of bacteria which are identical when studied by cultural and morphologic methods have been found to be composed of a number of groups which are only determined by their biologic differences. When studied by serologic methods these groups are found to be more or less distinct. This comparatively recent method of differentiating bacteria which are otherwise similar has been productive of important advances in the knowledge and in the treatment of infectious diseases. The meningococci and the pneumococci are familiar examples of this fact. Until the various biologic groups of the former were carefully investigated the serum treatment of meningitis due to this organism was much less satisfactory than it has since become. To a lesser extent and in a degree which up to the present has not been productive of such practical results, the typhoid-colon group has been subdivided into a number of serologic groups.

The hemolytic streptococci, then, are not unique among the bacteria. On the other hand, it might be assumed that distinct groups exist in respect to their biologic properties from the fact that various strains present differences in their cultural characteristics and from their parasitic nature. From the present study of a quite representative series of strains of this organism it would seem that this is so.

The value of agglutination reactions in the study of streptococci has been a disputed point. Thus Kligler[13] has expressed the belief that agglutination tests cannot be depended on in the classification of streptococci. On the other hand, Smith and Brown[14] have used this reaction to demonstrate the identity of different organisms isolated during the course of an epidemic.

However this may be, a practical and simple method of studying the agglutinability of hemolytic streptococci appears now to be available. The results obtained on the basis of agglutination tests have been checked, not only by bactericidal tests, but by protective experiments as well, in the living body. The fact that these last two methods which are less susceptible to erroneous interpretation corroborate the evidence obtained from agglutinations is strong evidence in favor of the reliability of this method in the classification of streptococci.

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[13] Jour. Infect. Dis., 1915, 16, p. 327.

[14] Jour. Med. Research, 1915, 31, p. 455.

The universality of the strains which form the basis of this study supports the assumption that they constitute a representative series. It can at least be assumed that a significant number of these strains fall into definite groups, a number so large that it cannot be disregarded in a consideration of the hemolytic streptococcus. The greatest value of the study of this series lies in a strong argument for the classification in the future of all infections due to this organism.

The finding of evidence that about 10% of the strains of this series show more or less relationship to other groups than the one to which they obviously belong points to the conclusion that the groups of the hemolytic streptococci as herein determined are not so clear-cut as the pneumococcus groups, but yet they appear to be somewhat more definite than the various strains of meningococci which grade into one another by a series of intermediate strains. The great majority of hemolytic streptococci seem to fall into these 3 fairly definite groups, as is clearly evidenced by the protection experiments with mice. Less evidence of an interrelationship is furnished by bactericidal experiments than by agglutination tests and animal inoculations show this to an even less degree.

It has been shown that these 3 groups constitute 93% of this series of 292 strains. The remaining 7% seem to represent strains which possess no biologic properties in common and which consequently cannot be grouped by the methods used in determining the relationship of the other 93%. They seem to be somewhat analogous in this respect to the strains of type IV pneumococci. Whether this is the case or whether they represent inagglutinable strains cannot be stated definitely at present. Judged from the agglutination test, they do not represent an intermediate group or groups, since none of the three group serums affects them in even the lowest titer.

A classification of the strains of this series by their pathologic source brings out several interesting points. The importance of epidemiologic evidence which may be gained from a study of epidemics due to hemolytic streptococci by the methods described is illustrated by the study of a small epidemic of sore throats and tonsillitis which occurred in a hospital ward and, as was shown, the great majority of infections were due to group 3 organisms, suggesting a common source. The comparison of the antemortem pneumonia cultures (sputum) with the series of lung cultures obtained at necropsy suggest a prognostic importance for infections with group 1, as compared with the other